Influence of Dithiocarbamates used for Phytosanitary Treatments on the Microelement Contents from Vineyard Soil in Tohani Region of Romania

CATALINA CALIN[1]*, GINA SCAETEANU (VASILE)[2], OCTAV PANTEA[1], LEONARD ILIE[2], ANDREEA BONDAREV[1]* , SONIA MIHAI[1]

[1]Petroleum-Gas University of Ploiesti, Romania, Chemistry Department, 39 Bucharest Av. Ploiesti, Romania

[2]University of Agronomic Science and Veterinary Medicine Bucharest, Romania, Soil Science Department, 59 Marasti Av., Bucharest, Romania

The influence of dithiocarbamates used for phytosanitary treatments of vineyard on the microelements contents from soil was studied in this work. It is well known that zinc and manganese levels in vineyard soils are increased by long-term application of fungicides. Dithiocarbamates, especially Mancozeb, are widely used to protect vegetal species against fungal diseases. The aim of this study was to establish if the levels of mobile forms of zinc and manganese increased hazardously after Mancozeb treatments at Tohani vineyard, from Prahova County, Romania. Having in view this purpose, it was analyzed soil samples from vineyard experimental plots that were treated exclusively with dithiocarbamates (Mancozeb) during year 2010. Experimental plots contain different varieties of vineyard: Feteasca Regala 1, Feteasca Regala 2, Sauvignon Blanc, Riesling Italian, Busuioaca de Bohotin and Feteasca Neagra. In this study was investigated the effects of Mancozeb on the metals mobility from soil samples. The analyses performed by Flame Atomic Absorption Spectrometry (FAAS) indicated that contents of mobile zinc and manganese increased after Mancozeb treatments, but the danger regarding the usage of this dithiocarbamate fungicide could be rather connected with its degradation products, than with metal contents. Treatments with fungicides containing Mancozeb, used in the spray program during growing season, usually provide Mn and Zn in tolerable doses and toxicity can result if the soil content in these elements is high, the soil reaction is acidic and if it rains after these treatments.

Keywords: dithiocarbamates, Mancozeb, vineyard, fungicides, Tohani

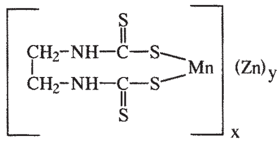
Dithiocarbamates are included in the class of fungicides and are widely used because they display a high broad-spectrum activity against fungal plant diseases. They are important organosulfur compounds that act as inhibitors of some enzymes (metal dependant and sulfhydryl) and as consequence strongly affect the biological systems. Dithiocarbamates are the main group of fungicides used to control approximately 400 pathogens of more than 70 crops and are registered in all EU member states and many other countries. [1,2]

These compounds could influence the content of microelements from soil because readily react with metallic ions, forming very stable complexes. Dithiocarbamates decompose in soils and this behavior could influence the content of available metals. Moreover, the literature surveys revealed that the stability of dithiocarbamate compounds in solution is strongly influenced by pH. In acidic medium, the compounds are cleaved at the nitrogen-carbon bond, leading to carbon disulfide and amines. In neutral and alkaline solutions, dithiocarmabate anions can be oxidized to compounds that still present features of dithiocarbamates [3].

According to literature reviews, the main environmental issue that appears after dithiocarbamates usage as fungicide is correlated with residues that are formed after degradation [4-6].

Ethylenethiourea and propylenethiourea are the most important degradation products of dithiocarbamates and they are suspected to cause pathogenic effects [7]. It was also proved that dithiocarbamates degradation products can cause tumors in thyroid, neuropathological effects and are suspected to be carcinogenic, mutagenic and teratogenic [7- 11].

Zinc and manganese concentrations in vineyard soils are increased generally by long term application of metal containing phytopharmaceuticals. The most significant source of zinc and manganese are nowadays dithiocarbamate based fungicides, as for example those that contain Mancozeb: Dithane M45, Dithane Neotec (fig. 1).



Mancozeb

Fig. 1. Mancozeb structure [manganese(2+) ethane-1,2-diylbis(dithiocarbamate) zinc ethane-1,2-diylbis (dithiocarbamate)]

Mobility and availability of manganese and zinc in soil is closely connected to soil reaction. It is known that the increase of soil acidity lead to the increase of the quantity of element that is available for plants [12].

Manganese and zinc are micronutrients that are involved in plant metabolism. So, manganese is important in activating enzymes, catalyzes the reduction of nitrate, and is a constituent of some enzymes that are responsible with respiration and protein synthesis. Manganese is involved in photosynthesis, in the water splitting system of photosystem II (or water-plastoquinone oxidoreductase) [13].

Zinc is an essential component of enzyme systems that are involved in many cellular processes, protein and auxines synthesis. [14]

* email: catalina.calin20@yahoo.com, Tel. + 40 - 244.573 171

The aim of our research was to study the evolution of manganese and zinc (mobile forms) from soil before and after phytosanitary treatments with dithiocarbamates (Mancozeb), during and after remanence period.

The purpose of this study was also to asses the possible detrimental effects the phytosanitary treatments might have on the local environment. In order to fulfill our objectives, soil samples were collected from experimental plots from Tohani vineyard (0-20 cm depth), during the year 2010. During 2010, on these experimental plots were not applied other chemical treatments, except Mancozeb products (Dithane M45 and Dithane Neotec).

Experimental part

Material and methods

Description of the area

Tohani is a locality in Prahova County, Romania. From geographical point of view, Tohani is located in a downy area covered by the Curvature Sub-Carpathians and it became known over time because of the important wine-growing areas (figs. 2a, 2b). Placed in the heart of Dealu Mare vineyard, Tohani area is a well-known place on the already famous "wine road". It is also the beneficiary of ideal conditions for grapes and vine harvesting, being surrounded by a favorable microclimate, that allows the grapes to ripen 10 days earlier than the vineyards in the neighborhood. It is notorious that Vineyard "Great Hill", called "Motherland of Red Wines" in Southern Carpathians, is the Romanian wine area with climatic conditions very similar to the Bordeaux region [15]. The climate is temperate continental, with cold winters and hot summers. The average annual temperature is 11.3°C and the recorded mean annual precipitation is 642 mm.

In Tohani area, the most common soils are Cambisol, Luvosol and Regosol. These are soils with a medium texture in the upper horizon and they are characterized by moderate natural fertility. The particle size distribution of soil from plotted area (Tohani) indicated a medium clay-loam texture (table 1).

The soil is characterized by a very low coarse sand content (1.4%), high percentage of fine sand, such as 35.8% and high clay content (40.2%).

Table 1

CHARACTERISTICS OF SOILS (0-20 CM DEPTH) FROM TOHANI AREA, ROMANIA

Granulometric fractions (mm)		% from soil material
Coarse sand	2.0-1.0	0.2
	1.0-0.5	0.2
	0.5-0.2	1.0
	2.0-0.2	**1.4**
Fine sand	0.02-0.01	2.8
	0.01-0.05	0.1
	0.05-0.02	32.9
	0.2-0.02	**35.8**
Silt	**0.02-0.002**	**22.6**
Clay	**<0.002**	**40.2**
Textural class	**medium clay-loam**	

Soil analyzed has a heavy texture that may have unfavorable consequences on main physical properties (bulk density, soil porosity, permeability, penetration resistance etc.).

Soil sampling

The investigation was carried out during the year 2010, on vineyard plots from the domain Tohani-Dealu Mare, a 20 year-old winemaking heritage.

Soils from plots with different varieties of vine (Feteasca Regala, Sauvignon Blanc, Riesling Italian, Busuioaca de Bohotin and Fetească Neagra) were analyzed. The analyses were performed for soil samples collected from 0-20 cm. There were collected six soil samples each time (before/after each treatment). Before proceeding to analyses, the soil samples were air dried, lightly grounded and sieved through 2 mm nylon sieve.

Chemical analyses and apparatus

The agrochemical parameters of soil samples were investigated: *p*H, mobile forms of phosphorus and potassium. The soil reaction (soil *p*H) was carried out through potentiometric method, in an aqueous suspension, 1:2.5 (w/v).

Mobile phosphorus content was evaluated by spectrophotometric molybdenum blue method, using ammonium acetate-lactate (AL) as extracting solution, at *p*H 3.7 (Egner-Riehm-Domingo method). The mobile form of potassium was quantified in the same extract (AL) by flame photometry.

A literature survey presents many extraction methods for mobile forms of metals from soil, sediments and sludge [16-21], but in agrochemical analyses is usually adopted method developed in [21].The mobile forms of manganese and zinc were extracted according to above mentioned method [21] and were quantified by Flame Atomic Absorption Spectroscopy (FAAS).

A soil sample of 10 g was treated with 50 mL extractive solution (EDTA 0,01M and CH_3COONH_4 1N, at *p*H 7) then it was stirred for 2 h and filtered off [21]. The resulted extract was used to evaluate the mobile forms of zinc and manganese. The analyses were performed with a Varian AA240 FS apparatus, in air-acetylene flame and the analytical lines were selected at 213.85 nm for zinc and 279.48 nm for manganese.

All analyses were performed in duplicate and the reported value is the average. The calibration curve for selected metals is linear for the studied ranges and was plotted by running different concentrations of standard solutions that were prepared using the same extraction solution used for the soil samples. (fig. 2, 3). A stock solution of 1000 ppm zinc/manganese provided by Merck was used to prepare the standards for calibration curve.

All the materials and vessels were kept for at least 24 h in a plastic container filled with HNO_3 10% (v/v) and then washed for several times with deionized water. All reagents used in this study were analytical grade. Stock standard solutions were freshly prepared from standard solutions provided by Merck. The ultra pure water used for samples analytic preparation was obtained from a Millipore Simplicity purification system.

Vineyard treatments

Dithane M45 (1.6 kg/ha, 80% Mancozeb) was applied at various intervals: at 17.06.2010, 28.06.2010 and 08.07.2010, for varieties Feteasca Regala 1 and Feteasca Regala 2; the soil samples were collected at 14.07.10.

Other treatment with Dithane M45 (1.6 kg/ha, 80% Mancozeb) was applied at 21.07.2010 and the soil samples were taken at 22.07.2010.

For varieties Sauvignon Blanc, Riesling Italian, Busuioaca de Bohotin and Feteasca Neagra it was applied at 18.05.2010 one treatment with Dithane Neotec (2 kg/ha, 75% Mancozeb); six soil samples/each type were collected at 14.07.2010 for analysis.

Results and discussions

Mobile phosphorus (P_{AL}) from soil ranges between 54-67 mg/kg for the varieties Feteasca Regala 1 and Feteasca Regala 2, this meaning a low content for vineyards; mobile

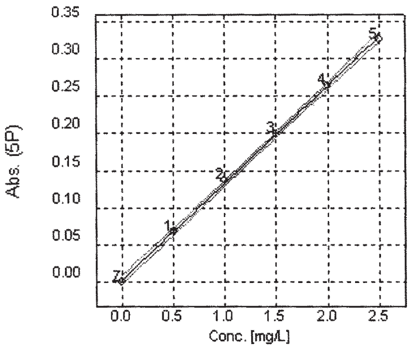


Fig. 2. Calibration curve for Mn; R^2(adj.): 0.9991

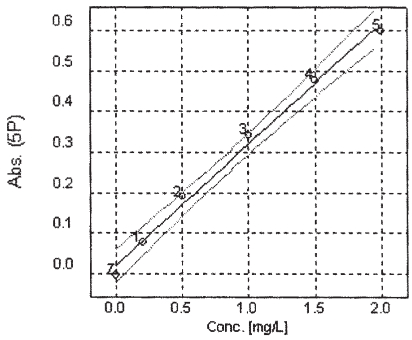


Fig. 3. Calibration curve for Zn; R^2(adj.): 0.9981

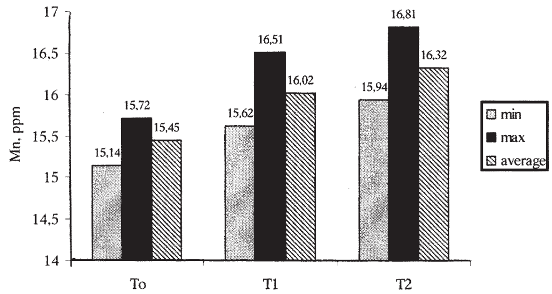


Fig. 4. Manganese content before (T_0) and after (T_1, T_2) Mancozeb treatments, for varieties Feteasca Regala 1

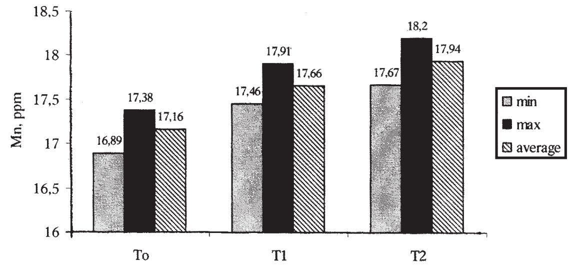


Fig. 5. Manganese content before (T_0) and after (T_1, T_2) Mancozeb treatments, for varieties Feteasca Regala 2

potassium levels (K_{AL}) ranges between 273-312 mg/kg for all soil samples, these values indicating a content which does not affect the vineyards.

For Sauvignon Blanc, Riesling Italian, Busuioaca de Bohotin and Feteasca Neagra varieties, mobile phosphorus was between 48-71 mg/kg, which represents also a weak content for vineyards. The potassium (K_{AL}) concentration was found between 281-334 mg/kg, this suggesting a good potassium content for intensive vineyards.

The soil reaction for vine varieties Feteasca Regala 1 and Feteasca Regala 2, before and after treatments, was found to be slightly alkaline (table 2, table 3). The soil *p*H for Sauvignon Blanc, Riesling Italian, Busuioaca de Bohotin and Feteasca Neagra was slightly acidic (table 4, 5).

Regarding the mobile manganese from soil, it was found that for varieties Feteasca Regala 1 and Feteasca Regala 2 the content of this microelement is medium level provision before and after treatments (figs. 4, 5), according to limits set by [21].

It was observed that in the case of Feteasca Regala 1, mobile form of manganese increased (as average) with 3.68% after treatment T_1, total increase being 5.63% (after T_1+T_2). For Feteasca Regala 2, after T_1, manganese increased with 2.91%, with a total increase of 4.54% (after T_1+T_2).

Mobile zinc concentration was found to be low level provision (< 1.5 ppm) before and after treatments (fig. 5). Anyway, according to literature [12, 22-24], zinc concentration in soil increases annually from 0.5 to 1.0 ppm, due to the use of fungicides and fertilizers that contain zinc. According to Romanian Ministry of Waters, Forests and Environment Protection (Law 756/1997), normal value for zinc total content in soil is 100 ppm, meanwhile alert value for sensitive soils is 300 ppm [25].

For Feteasca Regala 1, zinc mobile content recorded an increase of 27.47% after T_1 with a total increase of 32.96% (after T_1+T_2). For Feteasca Regala 2, the increase of zinc mobile content was higher than for Feteasca Regala 1: 38.20% (after T_1) and 59.55% (after T_1+T_2).

Even if Mancozeb contain manganese and zinc, it is possible that due to slightly alkaline reaction, these elements to adopt insoluble and inaccessible forms. Mobility of zinc and manganese is strongly related with soil reaction: increased acidity leads to a higher quantity of accessible elements to plants; meanwhile, alkaline reaction causes the transformation of soluble forms of elements into insoluble ones.

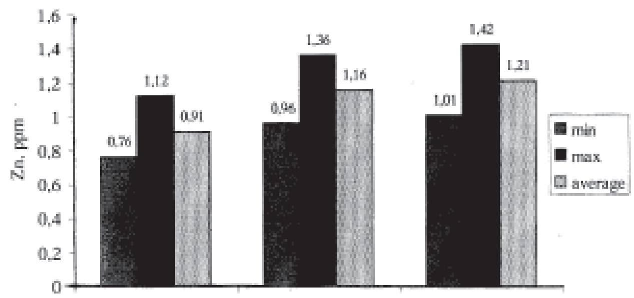


Fig. 6. Zinc content before (T_0) and after (T_1, T_2) Mancozeb treatments, for varieties Feteasca Regala 1

Table 2

MOBILE FORM OF MANGANESE AND *p*H VALUES FOR SOIL SAMPLES BEFORE AND AFTER PHYTOSANITARY TREATMENTS WITH DITHIOCARBAMATES (MANCOZEB)

Soil samples	Treatment	pH	Mn, ppm (min-max)	$\bar{X}$ ± SD	CV, %
Feteasca Regala 1	T_0	7.24	15.14-15.72	15.45±0.24	1.58
	T_1	7.38	15.62-16.51	16.02±0.34	2.12
	T_2	7.34	15.94-16.81	16.32±0.28	1.77
Feteasca Regala 2	T_0	7.32	16.89-17.38	17.16±0.18	1.07
	T_1	7.41	17.46-17.91	17.66±0.15	0.89
	T_2	7.46	17.67-18.20	17.94±0.16	0.90

(results are presented as average ± standard deviation (SD) pentru n=6), CV(%)=coefficient of variation)

***T_0** - samples collected before treatments (6 soil samples); **T_1** - a sum of treatments with Dithane M45 (1.6 kg/ha, 80% Mancozeb) applied at 17.06.2010, 28.06.2010, 08.07.2010; samples were collected at 14.07.10 (6 soil samples); **T_2** - one treatment with Dithane M45 (1.6 kg/ha, 80% Mancozeb) applied at 21.07.2010; samples were collected at 22.07.2010. (6 soil samples)*

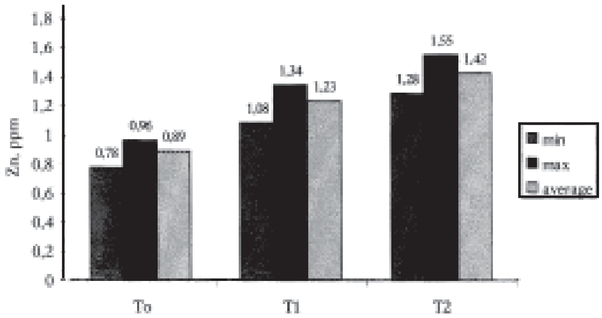


Fig. 7. Zinc content before (T_0) and after (T_1, T_2) Mancozeb treatments, for varieties Feteasca Regala 2

Table 3
MOBILE FORM OF ZINC AND *p*H VALUES FOR SOIL SAMPLES BEFORE AND AFTER PHYTOSANITARY TREATMENTS WITH DITHIOCARBAMATES (MANCOZEB)

Soil samples	Treatment	pH	Zn, ppm (min-max)	$\bar{X} \pm SD$	CV, %
Feteasca Regala 1	T_o	7.24	0.76-1.12	0.91±0.14	16.23
	T_1	7.38	0.96-1.36	1.16±0.16	14.24
	T_2	7.34	1.01-1.42	1.21±0.14	12.06
Feteasca Regala 2	T_0	7.32	0.78-0.96	0.89±0.07	7.93
	T_1	7.41	1.08-1.34	1.23±0.12	10.42
	T_2	7.46	1.28-1.55	1.42±0.09	6.80

(results are presented as average ± standard deviation (SD) pentru n=6), CV(%)=coefficient of variation)

T_o *- sample collected before treatments (6 soil samples);* ***T_1*** *- a sum of treatments with Dithane M45 (1.6 kg/ha, 80% Mancozeb) applied at 17.06.2010, 28.06.2010, 08.07.2010; samples were collected at 14.07.2010 (6 soil samples);* ***T_2*** *- one treatment with Dithane M45 (1.6 kg/ha, 80% Mancozeb) applied at 21.07.2010; samples were collected at 22.07.2010 (6 soil samples)*

Table 4
MOBILE FORMS OF MANGANESE AND*p*H VALUES FOR SOIL SAMPLES BEFORE AND AFTER PHYTOSANITARY TREATMENTS WITH DITHIOCARBAMATES (MANCOZEB)

Soil samples	Treatment	pH	Mn, ppm (min-max)	$\bar{X} \pm SD$	CV, %
Sauvignon Blanc (SB)	T_o	6.53	17.97-18.55	18.29±0.17	0.98
	T_1	6.67	18.86-19.32	19.08±0.17	0.90
Riesling Italian (RI)	T_0	6.65	29.62-30.22	29.85±0.24	0.80
	T_1	6.71	30.88-31.66	31.22±0.32	1.04
Busuioaca de Bohotin (BB)	T_0	6.74	24.65-25.24	24.92±0.17	0.69
	T1	6.79	25.03-25.76	25.34±0.25	1.00
Feteasca Neagra (FN)	T_0	6.68	25.66-26.12	25.85±0.17	0.65
	T1	6.65	26.29-27.22	26.72±0.29	1.11

(results are presented as average ± standard deviation (SD) pentru n=6), CV(%)=coefficient of variation)

T_o *- sample collected before treatments (6 soil samples);* ***T_1****- treatment with Dithane Neotec (2 kg/ha, 75% Mancozeb) applied at 18.05.2010; samples were collected at 14.07.2010 (6 soil samples).*

Table 5
MOBILE FORMS OF ZINC AND *p*H VALUES FOR SOIL SAMPLES BEFORE AND AFTER PHYTOSANITARY TREATMENTS WITH DITHIOCARBAMATES (MANCOZEB)

Soil samples	Treatment	pH	Zn, ppm (min-max)	$\bar{X} \pm SD$	CV, %
Sauvignon Blanc	T_o	6.53	1.46-1.72	1.58±0.11	7.48
	T_1	6.67	1.61-1.93	1.71±0.13	7.73
Riesling Italian	T_0	6.65	1.68-1.93	1.82±0.09	4.96
	T_1	6.71	1.84-2.26	2.03±0.17	8.52
Busuioaca de Bohotin	T_0	6.74	1.66-1.84	1.71±0.07	4.41
	T1	6.79	1.94-2.22	2.10±0.13	6.48
Feteasca Neagra	T_0	6.68	1.61-1.74	1.68±0.05	3.07
	T1	6.65	1.82-2.01	1.92±0.07	3.62

(results are presented as average ± standard deviation (SD) pentru n=6), CV(%)=coefficient of variation)

T_o *- sample collected before treatments (6 soil samples);* ***T_1****- treatment with Dithane Neotec (2 kg/ha, 75% Mancozeb) applied at 18.05.2010; samples were collectedat 14.07.2010 (6 soil samples).*

The results demonstrated that the soil reaction was slightly acidic before and after treatments for Sauvignon Blanc, Riesling Italian, Busuioaca de Bohotin and Feteasca Neagra varieties (tables 4, 5); this could explain the middle content for manganese and zinc. A moderate increase of manganese and zinc contents was observed and *p*H was still slightly acidic, after treatments with Dithane Neotec.

Manganese content (mobile form) increase as follows: Busuioaca de Bohotin (1.68%) < Feteasca Neagra (3.36%) < Sauvignon Blanc (4.32%) < Riesling Italian (4.59%), meanwhile zinc content (mobile form) increase as follows: Sauvignon Blanc (8.22%) < Riesling Italian (11.53%) < Feteasca Neagra (14.28%) < Busuioaca de Bohotin (22.8%).

Manganese and zinc are very important agrochemical microelements, but exceeding certain limits and in correlation with soil reaction, organic matter content and with climate (precipitation after treatments, excessive humidity), environmental problems could appear.

Our research revealed that hazard concerning dithiocarbamates, especially Mancozeb [manganese(2+) ethane-1,2-diylbis(dithiocarbamate) zinc ethane-1,2-diylbis(dithiocarbamate)] is rather correlated with its degradation products, than with manganese and zinc pollution. This represents a starting point for further studies that are related with identification and quantification of degradation products.

Some laboratory studies [26] suggest that pesticide degradation rates and mechanisms are similar in tropical and temperate soils, but there are studies that indicated that in tropical soils dissipation occurs more rapidly than under temperate conditions [26, 27]. As consequence in the case of tropical soils, constant use of Mancozeb (over 10 years) led to high accumulation of manganese in these types of soils; meanwhile zinc total content presented no

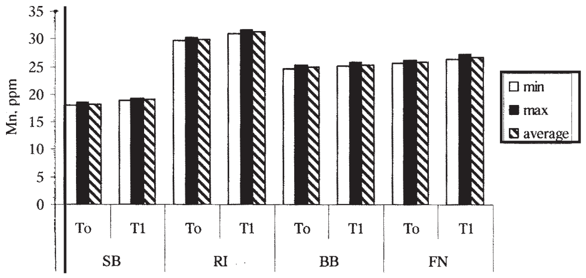


Fig. 8. Mobile forms of manganese for soil samples before (T_0) and after (T_1) phytosanitary treatments with dithiocarbamates (Mancozeb)

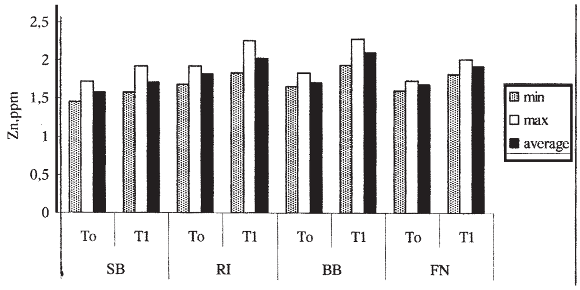


Fig. 9. Mobile forms of zinc for soil samples before (T_0) and after (T_1) phytosanitary treatments with dithiocarbamates (Mancozeb)

significant accumulation [26]. The presence of ethylenethiourea was also evidenced in water used for human consumption [26], this meaning that application of dithiocarbamates suppose existence of management strategies that lead to decrease the use of such fungicides.

Conclusions

The influence of dithiocarbamates used for phytosanitary treatments of vineyard on the microelements contents from soil was studied in this work.

The aim of this study was to establish if the levels of mobile forms of zinc and manganese increased hazardously after Mancozeb treatments on experimental soil plots from Tohani vineyard (Prahova County). Experimental plots contain different varieties of vineyard: Feteasca Regala 1, Feteasca Regala 2, Sauvignon Blanc, Riesling Italian, Busuioaca de Bohotin and Feteasca Neagra.

The analyses performed by FAAS indicated that contents of mobile zinc and manganese increased after Mancozeb treatments, but the danger regarding the usage of this dithiocarbamate fungicide could be rather connected with its degradation products, than with metal contents. The degradation products of Mancozeb present carcinogenic effects and they could contaminate soil, grapes, and also wine as well.

It was observed that the manganese and zinc levels increased slightly after treatments for the varieties Feteasca Regala 1 and Feteasca Regala 2, but provision levels (according to limits set in [21]) remained middle and respectively, low. In this case, soil reaction was initially slightly alkaline and after treatments it remained in the same range, this being an explanation for the contents of manganese and zinc.

The manganese and zinc levels before treatments with Dithane Neotec was middle for the varieties: Sauvignon Blanc, Riesling Italian, Busuioaca de Bohotin and Feteasca Neagra and an increase was recorded after treatment; the soil reaction was slightly acidic in this case and this explains why the concentrations of manganese and zinc where higher.

The treatments with fungicides containing Mancozeb used in the spray program during growing season usually provide manganese and zinc in tolerable doses and the toxicity could appear if the soil content in these elements is high, the soil reaction is acidic and also, if it rains after treatments. This research represents a starting point for further studies correlated with identification and quantification of hazardous degradation products.

References

1.BLASCO, C., FONT, G., PICO, Y., Journal of Chromatography A, **1028**, 2004, p. 267.
2.*** Food Safety. Pesticide Residues, European Commission, Brussels, 2002, http://ec.europa.eu/food/fs/rc/index_en.html
3.MILANOVA, E., ELLIS, S.M., SITHOLE, B.B., International Environmental Conference & Exhibit, **1998**, p. 173.
4.BANERJEE, K.,OULKAR, D.P, ADSULE, P.G.,Acta Horticulturae, **785**, 2008, p. 409.
5.BASA CESNIK, H., GREGORCIC, A., VELIKONJA BOLTA, S.., KMECL, V., Food Additives and Contaminant, **23** (2), 2006, p.164.
6.CALDAS, E.D., MIRANDA, M.C.C., CONCEICAO, M.H., DE SOUZA, L.C.K.R., Food and Chemical Toxicology, **42,** 2004, p.1877.
7.VETTORAZZI, G., ALMEIDA, W.F., BURIN, G.J., JAEGER, R.B., PUGA, F.R., RAHDE, A.F., REYES, F.G.,CHVARTSMAN, S., Teratogen. Carcinogen.Mutagen, **15**, 1995, p. 313.
8.GEORGE, J., SHUKLA, Z., Journal of Proteomics, **74,** 2011, p.2713-2722.
9.SZOLAR, O.H.J., Analytica Chimica Acta**, 582,** 2007, p. 191.
10.KAZOS, E.A., STALIKAS, C.D., NANOS, C.G., KONIDARI, C.N., Chemosphere, **68,** 2007, p.2014.
11.FAO/WHO. Pesticide residues in food. Evaluations, 1993, Part II-Toxicology. Joint FAO/WHO Meetingon Pesticides Residues, Geneva, Switzerland.
12.WEINGERL, V. , KERIN, D., Acta Chim.Slov., **47,** 2000, p.453.
13.MILLALEO, R., REYES-DIAZ, M., IVANOV, A.G., MORA, M.L., ALBERDI, M., J.Soil Sci. Plant.Nutr. **10** (4), 2010, p.476.
14.HACISALIHOGLU, G., HART, J.J., KOCHIAN, L.V., Plant Physiology, **125**, 2001, p.456.
15.OPREA, M.C., Petroleum-Gas University of Ploiesti Bulletin, **LXII** (2), 2010, p.116.
16.VASILE, G. G., Monitorizarea concentraţiilor speciilor metalice mobile din soluri şi sedimente antropizate. Trasabilitatea măsurătorilor analitice de metale mobile în soluri şi sedimente. Teza de doctorat, Universitatea Bucureşti, Facultatea de Chimie, 2009.
17.VASILE, G., CRUCERU, L., Journal of Environmental Protection and Ecology **8(4)**, 2007, p.801.
18.VASILE, G., CRUCERU, L., PETRE, J., TANASE, I.Gh., Rev. Chim. (Bucharest), **58**, no. 12, 2007, p.1332.
19.VASILE, G., TANASE, I.GH., Rev. Roum. Chim., **53**, no. 11, 2008, p.1041.
20.ETTLER, V., MIHALJEVIC, J., SEBEK, O., GRYGAR, T., Analytica Chimica Acta **602**, 2007, p.131.
21.LĂCĂTUŞU, R., KOVACSOVICS, B., GÂŢĂ, GH., ALEXANDRESCU, A., Utilisation of ammonium acetate – EDTA by simultaneous extraction of Zn, Cu, Mn and Fe from soil. Pub. SNRSS 23B, 1-11 (in Romanian), 1987.
22.*** http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/93_rep/Report1993.pdf
23.MULKEY, M.E., The grouping of a series of dithiocarbamate pesticides based on a common mechanism of toxicity. U.S.Environmental Protection Agency, Office of Pesticide Programs, Office of Prevention, Pesticides and toxic substances, Washington, DC, 2001.
24.CĂLIN, C., SCĂEEANU, G., PELE, M, ILIE, L., PÂNTEA, O. BOMBOS, D., Rev. Chim. (Bucharest), **63** , no. 10, 2012, p. 1062.
25.*** Romanian Ministry of Waters, Forests and Environment Protection - Law 756/1997.
26.GEISSEN, V., RAMOS, F., BASTIDAS-BASTIDAS, P., DIAZ-GONZALES, G., BELLO-MENDOZA, R., HUERTA-LWANGA, E., RUIZ-SUAREZ, L.E., Bull. Environ. Contam. Toxicol., **85**, 2010, p.407.
27.RACKE, K.D., SKIDMORE, M., HAMILTON, D.J., UNSWORTH, J.B., MIYAMOTO, J., COHEN, S.Z., Appl.Chem., **69**, 1997, p.1349.

Manuscript received: 7.03.2013